

AN AVIAN TUMOR IN ITS RELATION TO THE TUMOR PROBLEM.

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The behavior of malignant tumors has stimulated many hypotheses as regards their causation and yet in some of its phases has appeared to disprove all. The suggestions afforded by other normal or pathological growth processes lead in numerous and diverse directions. Today, despite an immense accumulation of data, the solution of the tumor problem waits upon fresh findings; and to foretell the line of research which will yield these findings has not seemed possible.

The successful transmission of neoplasms of the lower mammals, a few years ago, seemed at first to carry with it the immediate solution of the problem. But this did not prove to be the case. In order to obtain a tumor-strain for investigation, an animal with a "spontaneous" tumor was required; and the transmission of the growth was soon found to involve a transplantation process,—as genuine a transplantation as that of skin or other normal tissue. A new tumor in the strict sense was not thus engendered, but to a portion of the old another host was given. All efforts to separate out a cause for the neoplasm or to transmit it by other means than by graft of the living neoplastic cells were unsuccessful. The consistently negative results of such work, together with the general behavior of the transplanted neoplasms, have led many investigators to forego the idea of an extrinsic cause for malignant tumors in general and to attribute them to some inherent cell-perversion, or else to a cell-deangement precipitated by factors temporarily active. But it may be pointed out that the basis for such a conclusion so far as it rests upon experiment, rests upon work with the tumors of few species. Those of the rat and mouse have been employed almost exclusively.

The findings here to be presented were obtained in the study of a malignant tumor of the chicken, which closely resembles in its general characters the mammalian neoplasms, including those of man. That such growths exist has been generally recognized; and their status as true tumors has been established. But like the neoplasms of other birds, of reptiles, amphibians, and many mammals, they have remained almost unutilized for research. Our tumor of the fowl proved transplantable and has thus far been observed in several hundred chickens. It is a connective-tissue growth, a spindle-celled sarcoma. From its tissue there has been isolated a causal agent, ultramicroscopic in some, perhaps in all, of its forms, and undoubtedly a living organism. Though the agent gives rise to the sarcoma, and accompanies the growth, it does not take any obvious share in the disease phenomena. These are referable to the behavior of the neoplastic cells, a point now to be illustrated.

The original sarcoma arose in a young fowl of pedigree, pure-blood stock, and its transplantation was successful only in this fowl's blood-relations. A similar peculiarity has been often observed on the transplantation of normal tissues, but it has not been noted in association with the transmission of diseases caused by a parasite. After propagation in several successive hosts, the sarcoma became less precise in its demands and could be transplanted to non-related fowls of the same variety. But like certain delicate tumors of mammals it was for a long time transmissible only within the limits of this variety, and at the latest test still grew most readily in such hosts. All attempts to transmit it to animals of other species have failed.

A transplantation of neoplastic tissue is involved in the growth's transmission under ordinary circumstances, and only by special means has it been shown to be unnecessary. Ordinarily when bits of the sarcoma are placed in a new and susceptible host they survive, are vascularized, and proliferating, form a new tumor. The multiplication of the implanted cells obviously suffices to produce the neoplasm. In the histological pictures there is no indication that the elements of the host ever become incorporated as true neoplastic tissue. No tumor arises in hosts so unfavorable to the engrafted tissue that it dies.

The growth consists of spindle-shaped cells supported by a scanty, vascular framework; and the sole differentiation which these cells undergo is to an attenuated form with the production of a few intercellular fibrils. In the disposition of the cell-strands and bundles there is no suggestion of focal arrangement such as frequently indicates, in the case of the granulomata, the presence and position of an exciting cause; while at the growth's borders a cellular reaction is practically absent. The tumor elements multiply rapidly by mitosis and amitosis, and the neoplasm grows, not only by expansive enlargement but also through an active invasion and replacement of the normal structures by tumor cells. In the course of the invasion tumor cells frequently penetrate the walls of blood or lymph-vessels, and are freed in the circulation. By their transportation, lodgment and growth secondary sarcomata are caused at points distant from the primary mass. This important characteristic of tumors in general has been placed beyond doubt as regards the avian growth by means of direct experimentation. The host, which at first seems unaffected by the tumor, emaciates as the growth increases in size, and, if it escapes intercurrent processes eventually dies in coma.

The conditions which determine the success or failure of the sarcoma when transplanted to a new individual are in general referable, as are those of its behavior and dissemination, to the sarcoma cells as such. The influence of variety of the host and of blood-relationship has already been referred to. Young hosts prove most favorable, as for all transplantable tissues, normal or neoplastic. Hosts which are ill of causes that involve emaciation are relatively unfavorable, a circumstance noted in its relation to mammalian growths by other workers, and especially interesting because these hosts are more susceptible, as a rule, to the frankly infectious processes. A certain proportion of hosts, although of the proper variety for the tumor's growth, manifest a resistance such that it does not develop when implanted in them; while others in which the growth has developed and retrogressed are completely resistant, for a time at least. Similar types of resistance have already been demonstrated for the tumors of rats and mice. Furthermore they

have been elicited against a normal tissue capable of growth on transplantation (embryonic tissue).

Taken together the foregoing traits identify the chicken sarcoma as a typical malignant tumor. In them there is nothing to suggest the presence of a parasitic cause for the disease, but much that has been held to favor the view of an intrinsic cell-derangement.

For the first attempts to separate out the sarcoma's cause filtration was employed. The tissue of a rapidly growing tumor was ground with sand, taken up in Ringer's solution, shaken for some time, centrifugalized, and the supernatant fluid was passed through a Berkefeld filter which held back small bacteria. The injection of a few cubic centimeters of the limpid fluid thus obtained gave rise to the characteristic spindle-celled growth in fowls previously normal; and this growth was capable of further transplantation through an indefinite series of hosts. More recently the causative agent has been differentiated from the living tumor cells by drying, by glycerinization and by repeated freezing and thawing,—processes which the tumor cells fail to survive. It remains active for many months in dried sarcomatous tissue, and for at least one month in tissue placed in 50 per cent. glycerin. It is quickly rendered innocuous by temperatures above 53° C., by 50 per cent. alcohol, by 2 per cent. phenol, by saponin in high dilutions, by chloroform and toluol in the proportions which prevent bacterial growth during autolysis, and by autolysis itself. It will not pass through a dialyzing membrane, nor, in our experience, through a porcelain filter. These various features seem sufficient to identify it as a living organism in distinction from a ferment. The organism has never been directly observed in fresh or stained preparations; and the morphology of the individual tumor cells does not suggest its presence. Attempts to cultivate it *in vitro* have not as yet proven successful.

The neoplastic change brought about by the agent takes place slowly compared with the proliferation of the cells, once they have become sarcomatous. Growth of the tumor, dissemination, injury to the host, immune processes, all are referable to these cells suddenly endowed with new properties. The introduction into a susceptible fowl of a large amount of the filterable agent is not in itself

sufficient to cause a tumor. The development of a growth under these circumstances is conditional upon the presence of a cell-derangement, such, for example, as is produced by the injection of infusorial earth. Yet even when the element of cell-derangement has been supplied, and the agent injected in quantity, a considerable percentage of the fowls fail to develop a sarcoma. The nature of the factors responsible for this failure has not been determined. The importance of cell-derangement as a contributory cause of human sarcomata has long been recognized.

The chicken sarcoma is strikingly non-infective under ordinary conditions. During the last three years more than a thousand fowls, with or without the tumor, have been kept together in close quarters, yet no instance of natural transmission has been observed. An examination of numerous spontaneous chicken tumors from various sources has shown that the sarcoma is not epidemic. These facts find an explanation in the various factors by which the agent's action is conditioned.

In conclusion it should be stated that the experiments with the chicken sarcoma have not yielded a method whereby a causative agent can be separated from the tumors of rats and mice. But they clearly prove that the characteristics of malignant tumors in general are compatible with the presence of a living causative agent. Such a cause for them seems, indeed, far from improbable.

Note: Dr. James B. Murphy has shared, as joint author, in the work on the chicken sarcoma since the recognition of the latter's filterable cause; and more recently Dr. W. H. Tytler has aided in the study of some of the growth's problems.

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